STUDIES ON PLANT CANCERS III. THE NATURE OF THE SOIL AS A DETERMINING FACTOR IN THE HEALTH OF THE BEET, BETA VULGARIS, AND ITS RELATION TO THE SIZE AND WEIGHT OF THE CROWN GALL PRODUCED BY INOCULATION WITH BACTERIUM TUMEFACIENS[1]

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Little is known concerning either the effect on the size and the weight of beets, either spontaneously or artificially infected with crown gall, or the effect of the general vigor of the plant on its susceptibility to infection. Many problems of practical and theoretical importance along these lines remain to be solved. Among them are: (1) a more accurate evaluation of the effects of the crown-gall disease upon the final size of the infected beet and hence on the beet crop; (2) the cause of the greater susceptibility of the sugar beet as compared with other races to the crown-gall organism; (3) the breeding of beets to obtain strains immune to crown-gall infection and yet retaining the desirable marketable qualities; (4) the cytological difference between crown gall and "tuberculosis" of beets and the relation of the former to animal cancer; (5) the relation of the soil to the health of the beet and to the size of the crown gall that it will harbor when inoculated with *Bacterium tumefaciens*.

Up to the present very little evidence has been advanced by animal pathologists to show any definite relation between the physical condition and the virulence of cancer once it is started.

The purpose of this paper is to present some data on the last of the questions noted above; namely, the relation of the health of the beet to the size of the crown gall resulting from infection. Since the term "health" has but a relative meaning, we shall measure health in this case in terms of size and weight, the matters most important to the beet grower and without doubt fair indices of the vigor and normality of the beet plant.

Inasmuch as crown gall is analogous to animal cancer, as Smith (1911, 1912), Magnus (1918), Levine (1919, 1920), Levin and Levine (1920), and Jensen (1918) have pointed out, these results also have a bearing on the general question of feeding in cancer and a more specific relation to the question of the relation of general vigor and vitality to susceptibility.

The older literature concerning the crown gall of beets is too well known

[1] From the Department of Cancer Research of Montefiore Hospital, Dr. I. Levin, Chief.

to need mention here. Only those papers that deal with the problem at hand are reviewed.

Tumor-like growths on the different varieties of beets have been recognized in America and Europe for a long time. These growths occur sporadically. Destructive epidemics are not known. Smith, Brown, and Townsend (1911) have shown that at least the gall formation known as crown gall[2] of the beet is produced by *Bacterium tumefaciens*. They were able to isolate the organism from the tumors on the beet and to infect beets, causing the formation of new crown galls.

Townsend (1915), studying the crown gall of the sugar beet which appears spontaneously in the field, concluded that the galls have no marked effect upon the size of the beet. The largest as well as the smallest beets may become seriously affected, and it is impossible to know whether roots would be large or small if they had been free from the infection. The galls begin to appear when the beets are one fourth to one half grown, that is, about midsummer; and from that time on they may appear at any time until harvest, so that crown galls of various sizes and ages may be found on the beets.

Jensen (1918) in his investigation of tumor-like growths in plants studied both spontaneous crown galls from the common races of beets and those that he produced experimentally by inoculation. He concludes that in the case of the mangel wurzel the crown gall has no detrimental effect upon the growth of the plant. In the garden beet, the tumor attains only slight size. In the sugar beet, on the other hand, Jensen finds the disease resulting in enormous tumor-like formations, irregular knotty structures which in section exhibit a very irregular arrangement of the vascular bundles differing markedly from that in the normal portion of the root. Jensen does not report on the relative sizes of the galls and roots in the sugar beet, nor does he discuss the structural characters of the normal roots of the various races for possible suggestions as to the cause of the differences in the size and structure of the galls.

Materials and Methods

The cultivated garden varieties, Early Model, Egyptian Early, and Giant Mangel Wurzel of *Beta vulgaris* were studied in my experiments. A preliminary test of the effect of the soil on the size of the root and crown gall produced when the roots were inoculated with *Bacterium tumefaciens* was carried out in six-inch pots in which a mixture of Early Model and Egyptian Early seeds was planted. Four kinds of soil were used. Each kind was placed in 12 pots. The first group was filled with garden soil mixed with an abundance of manure that had been previously used for mushroom culture. The second contained a brown loam that was obtained

[2] "Tuberculosis" of beets is a tumor-like growth on beets produced by *Bacterium beticolum* (Smith, Brown, and Townsend, 1911).

from a neighboring lot, and the third, a combination of equal parts of garden soil and manure and of the loam used in the second. The fourth group was filled with a medium sand used for building purposes. Three seedlings were planted in each pot. The pots were placed in boxes, and the spaces between the pots were filled with hay. The boxes were then placed in the open where they were exposed to the light all day. When the tap roots began to appear, the soil was gently removed from one side of each root, and, with a needle dipped in a culture of *Bacterium tumefaciens*, the root was pricked five to fifteen times. The soil was then returned and the plants were not disturbed again until the middle of October. Then they were carefully removed from the soil and studied. In each group of pots, some plants were left uninoculated to serve as controls.

Field studies were made in three plots 12 x 25 feet each. The first two, "E" and "W," consisted of soil well worked and thoroughly mixed with an abundance of manure. The third, "SW," consisted of a coarse sandy soil never used before and unfertilized. In early May, the plots "E" and "SW" were planted with a mixture of Early Model and Egyptian Early. The rows were thinned out in June, and in the latter part of July, when the tap roots began to appear, they were inoculated in the manner described above with young cultures of *Bacterium tumefaciens*. Each inoculated plant was labeled. An equally large number of uninoculated plants were left growing among the inoculated ones to serve as controls. The plots were equally well exposed to sunlight for the greater part of the day. They were similarly worked and watered. A considerable number of inoculated plants were gathered from time to time and fixed for a cytological study to be reported on at a later time.

Plot "W" was sown with the Giant Mangel Wurzel seeds and treated as the plots "E" and "SW" were.

Relation of the Soil Quality to the Size and Weight of the Root and of the Crown Gall

We may consider first the experiments in which the beets were grown in pots as described above. The effect of the crown gall on the total beet crop grown under these conditions is only suggestive since the number of our experiments was relatively small. The relation of the size and weight of the root to the size and weight of the crown gall is the point with which we are specially concerned.

In this series of experiments, as noted, a mixture of seeds of the garden beet varieties, Early Model and Egyptian Early, were grown in 48 six-inch pots in four different kinds of soil. In the first group, made up of 12 pots, the soil consisted of a mixture of manure that had previously been used for mushroom culture and an equal quantity of garden soil. The second group of pots were filled with a brown silt loam, the third group contained equal parts of manure and brown silt loam, and the fourth contained a medium sand.

TABLE I. *Effect of the soil on the weights*[3] *of the garden beet, Beta vulgaris, vars. Early Model and Egyptian Early, the roots of which were inoculated with Bacterium tumefaciens. Growing in pots containing "A" garden soil and manure, "B" brown silt loam, "C" manure and brown silt loam, "D" medium sand*

No.	Pot A		Pot B		Pot C		Pot D	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
1......	31.75	3.30	81.40	5.40	38.30	3.00	36.80	3.60
2......	60.00	5.60	46.00	4.50	2.10	1.50	30.00	3.80
3......	113.60	11.80	37.10	3.00	46.10	4.70	38.60	3.60
4......	65.40	5.50	24.60	1.80	32.10	3.30	33.50	4.50
5......	38.60	4.60	51.00	4.10	10.00	1.20	23.70	2.70
6......	45.50	4.80	24.10	2.30	22.30	2.10	36.00	4.20
7......	45.00	5.30	26.00	2.70	36.50	4.40	37.70	4.30
8......	42.50	4.60	33.10	3.00	22.60	2.40	5.10	.90
9......	67.50	5.30	19.10	1.40	5.70	1.20	8.70	.90
10......	51.10	6.15	60.00	3.90	13.20	1.60	53.70	5.50
11......	71.10	7.10	66.60	7.50	9.50	1.00	13.70	1.90
12......	78.10	9.00	21.60	1.20	7.10	.80	9.00	2.10
13......	49.60	4.50	17.00	1.50	32.50	2.80	14.90	2.30
14......	25.50	2.20	9.50	1.60	51.00	5.00	24.00	2.10
15......	13.20	1.80	34.00	3.50	74.00	8.40	20.00	2.60
								corrections
16......	33.90	3.10	19.00		66.70	7.80	33.00	.80
17......	36.30	3.60	22.50	1.70	22.50	2.20	24.10	5.50
18......	22.40	2.50	27.50	2.60	55.80	4.40	3.70	3.60
19......	13.80	1.30	10.30	corrections	12.40	1.80	6.00	
20......					31.00	3.15		
21......					27.80	2.90		
22......				2.60	51.20	5.50		
23......				8.60	16.00	1.80		
24......				4.50	13.40	1.70		
25......				2.70	10.00	1.00		
Totals...	904.85	92.05	630.40	63.00	728.70	75.65	452.20	53.50
Averages	46.57	4.73	33.17	3.316	29.14	3.02	23.80	2.81

The plants, three to four in each pot, were inoculated as described above with *Bacterium tumefaciens* on July 20, 1920. On October 9, 1920, the plants were removed from the soil, and the sizes and weights of the roots grown in the different soils were compared.

Figure 1 shows the general development of the plants as they appeared in the pots and represents two average pots from each group of twelve, of the varieties Early Model and Egyptian Early. "A" represents the plants grown in the garden soil with an abundance of manure, "B" the plants in the brown silt loam, "C" the plants in brown silt loam and manure, and "D" the plants grown in medium sand.

The plants grown in pot "A" (garden soil plus manure) are much larger than those in pots "C" (brown silt loam and manure) and in "D" (sand), but the difference is not so marked between "A" (garden soil and manure) and "B" (brown silt loam). The leaves of the plants growing in pot "A" are somewhat larger and appear to be more numerous. While we

[3] Weights given in grams in all tables.

have no very reliable basis for measuring health or vitality quantitatively, it would be generally conceded that plants such as those represented in pot "A" are more vigorous and more healthy than those in pot "D."

On removing the plants from the soil, it was found that the size of the

FIG. 1, A, B, C, and D. Four pots filled with manure, brown silt loam, manure and brown silt loam, and sand, respectively.

leaves generally served as a good indication of the size of the roots. The roots whether inoculated or uninoculated were largest when grown in the soil and manuie, smaller when grown in brown silt loam or in the combination of the loam and manure, and smallest when grown in sand.

The size and weight of the crown gall in each case was directly proportional to the size and weight of the root, as will be shown below. It is seen that the weight of the entire plant grown in the four different soils shows that those plants which were grown in soil rich in organic material were well nourished and attained the greatest weights while those grown in sand weighed less. Table 1 gives the fresh and dry weights of the entire plants grown in the four different soils with their roots inoculated with *Bacterium tumefaciens*. Representative plants with their infected roots from each group are shown in figure 2. Figure 2, "A" shows an infected root of a plant grown in garden soil mixed with an abundance of manure. "B" was grown in brown silt loam and manure. "C" was grown in brown silt loam, and "D" was grown in sand. As shown in table 1, the infected plants grown in pots containing garden soil and manure attained a fresh weight of 46.57 g. and a dry weight of 4.73 g., while plants grown in brown silt loam

were next highest with an average weight when fresh of 33.17 g. and a dry weight of 3.32 g., and the plants grown in a combination of loam and manure fell slightly short of the weights attained in the loam alone. The difference, however, is slight. A striking difference is seen in the proportionate size

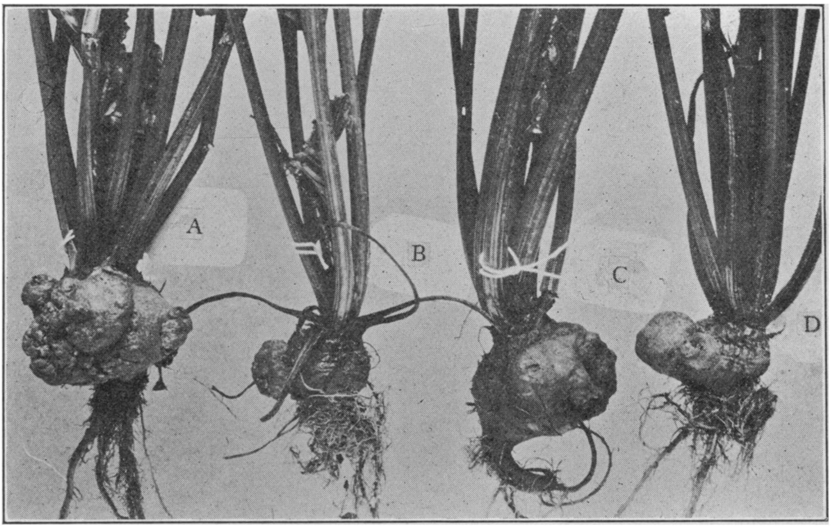

FIG. 2, A, B, C, and D. Roots of beets grown in pots filled with manure, manure and brown silt loam, brown silt loam, and sand respectively.

of the crown galls on the roots shown in figure 2, "A" and "D," and also in the average weights of the plants which are grown in the garden soil and sand respectively (see table 1). These results are quite striking and indicate rather clearly that the better nourished host responds to the parasitic infection more actively than the poorly nourished one, a fact which is generally recognized in animal pathology.

Better nutrition of the host does not tend to increased resistance to the growth of an inoculum of an animal tumor, but appears rather to have the contrary effect as shown by Ewing. It appears from these experiments that when the host is well fed the parasite is also well fed, it reproduces more actively, and produces a greater quantity of toxin which apparently calls forth relatively a greater hyperplasia of the host. The case is very clear here that normal development of the host rather favors the development of the crown gall. There is no evidence of increased resistance to the parasite in well grown as compared with poorly grown plants.

RESULTS OF THE FIELD EXPERIMENT

As mentioned above, similar seeds of *Beta vulgaris*, varieties Early Model and Egyptian Early, were planted in two plots, "E" and "SW," with an

area of 300 sq. ft. each. The first, "E," had been used during three previous seasons for root crops. The soil was well worked and abundantly treated with thoroughly rotted manure. The plot "SW" had never been used for a crop. It consisted of filled-in land. The soil was coarse and made up chiefly of coarse sand. No fertilizer was used on this plot. Beginning August 4 to 15, 1920, three hundred roots in each plot were inoculated with young cultures of *Bacterium tumefaciens* and labeled. A large number of beet roots growing among the inoculated plants were left undisturbed to serve as controls.

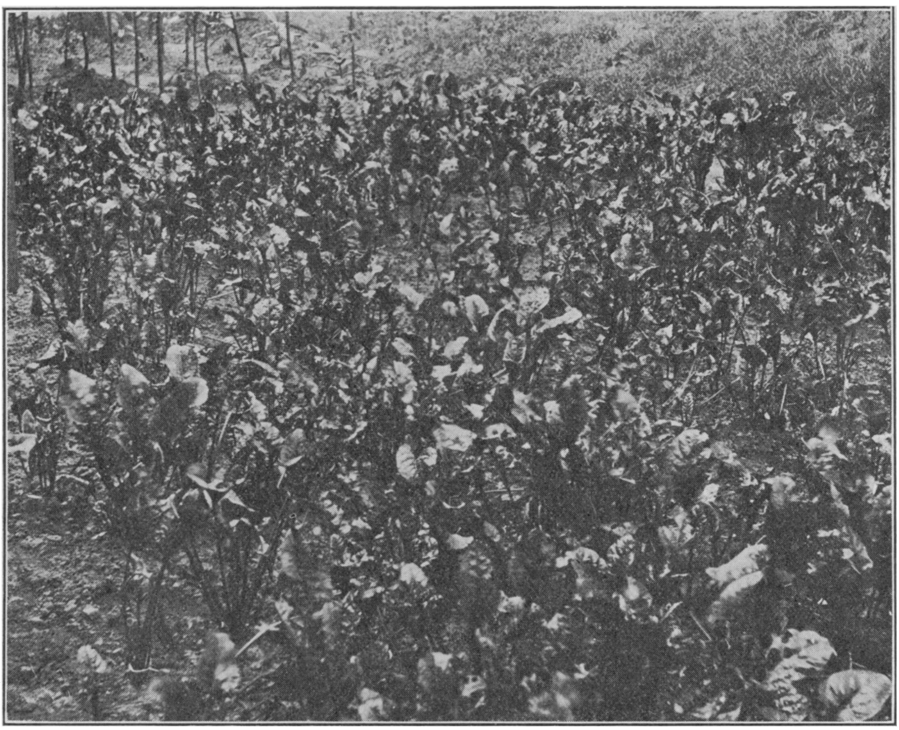

FIG. 3. A portion of plot "E" with the garden beet, one month after inoculating the root with *Bacterium tumefaciens*. The soil was well fertilized, and cultivated.

A difference in the size of the plants in the plots "E" and "SW" appeared very early. Figure 3 shows a portion of plot "E." The leaves are not only larger but more numerous. The rows appear to be indistinct owing to the fact that the leaves cover the ground. The plants in the plot at the time this picture was made had been inoculated one month. The difference between plots "E" and "SW" was even more pronounced at the time the crop was harvested.

Figure 4 shows part of the plot "SW" with the poor soil. The plants

are relatively small and the leaves few. A considerable number of plants in this plot died. Here, also, the figure represents the condition of the plants in the plot one month after inoculation. An examination at this time of the roots of a few plants from plot "E," on good soil, showed that the inoculated plants had produced crown galls which were about the size of small hickory nuts, while those on the roots of the largest plants in poor

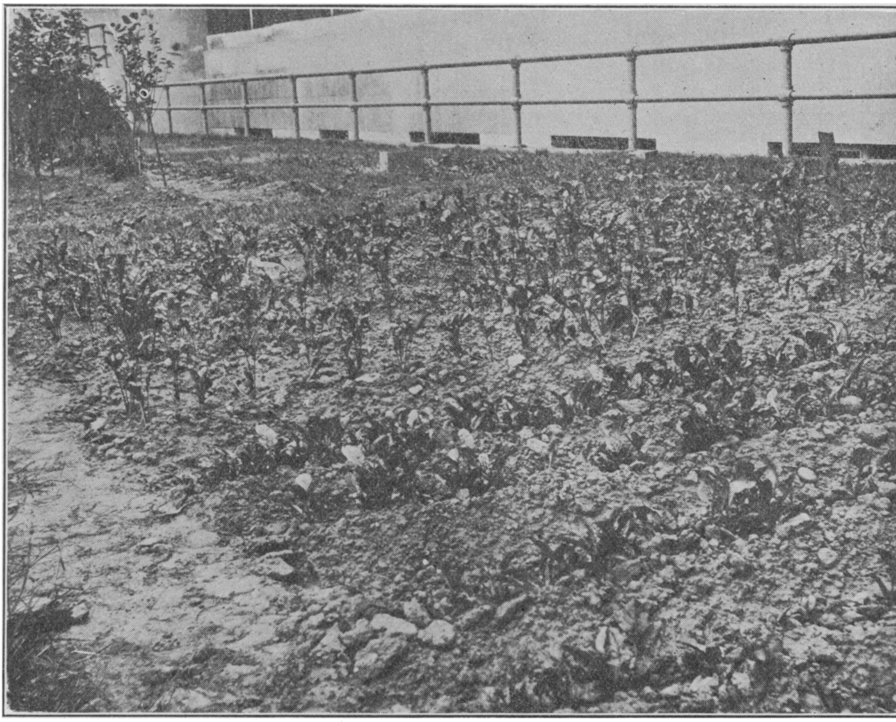

FIG. 4. A portion of plot "SW" with the garden beet, one month after inoculating the root with *Bacterium tumefaciens*; the soil was unfertilized and had never before been cultivated.

soil, plot "SW," were barely in evidence or at most had attained a size equal to that of a pea.

On examining the roots of the beets in October, when the entire group was harvested, the crown galls on the plants grown in the good soil, plot "E," were proportionately very large as compared to those on the roots of beets grown in plot "SW." Often the crown gall surrounded the entire upper portion of the root so that the normal contour of the root became indistinct. Figure 5 shows some typical roots with crown gall grown in good soil (plot "E").

The plants grown on poor soil (plot "SW") which were inoculated at about the same time as those growing in plot "E" were not only small,

but the crown galls were proportionately small as compared to the size of the root. Figure 7 represents a series of beet roots grown in plot "SW," photographed at maturity when the plants were harvested; in this series the smallest crown gall was smaller than a pea while the largest was about the size of a black walnut.

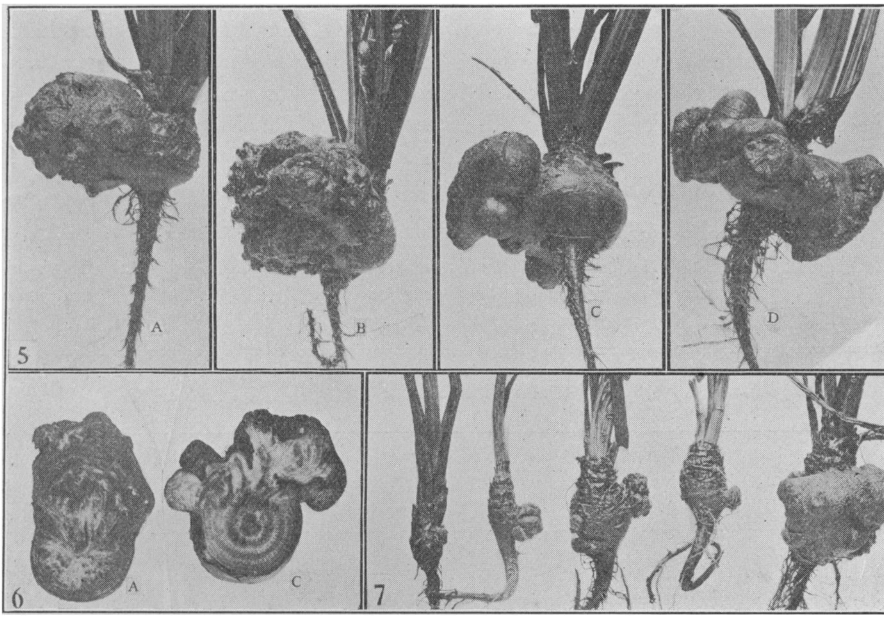

FIG. 5, A, B, C, D. A series of crown galls on the roots of the garden beet grown in fertilized soil; one month after inoculation. FIG. 6, A, C. Cross sections of the roots shown in figure 5, A and C. FIG. 7. A series of crown galls on garden beets grown in poor soil. Inoculation made August 11, 1920. Photographed October 16, 1920.

My observations on the appearance of the crown galls grown on these varieties of the garden beet are not in complete accord with those of Jensen, who claims that tumor-like growths on the different varieties of the garden beet differ from one another both in appearance and structure. In my cultures all the common types of crown gall appeared in each variety. Figure 5A represents the smooth type, 5B a warty gall, and 5C and 5D represent mixed types (smooth and warty), a form which Jensen does not recognize. In the mangel wurzel described below and shown in figure 9, we have similar types. The warty and the smooth types appear here as does the mixed type. It is of interest to note that crown galls of the smooth and warty types are also found in artificially produced crown galls on *Ficus elastica* (Levine, 1921.) The significance of these different types of crown gall is not yet perfectly understood.

In my observation of the galls on the Early Model and Egyptian Early

varieties, a number of smooth galls were found to be stemless as shown in figures 6A and 6C, which are cross sections of the beets shown in figures 5A and 5C respectively. The warty galls are as a rule stemless. The galls in the plants grown in plot "SW" were also of the three types, and the arrangement of the vascular bundles in the crown galls appears to depend upon the internal structure of the root before inoculation.

Shortly after the plants of the two plots "E" and "SW" were gathered they were carefully washed and weighed. They were loosely wrapped in separate pieces of paper and dried in a hot air oven at 100° C. for several days and then weighed again. The results are given in table 2. Columns 1 and 2 give the fresh and dry weights of the normal plants from plot "SW," that is, in the unfertilized soil.

TABLE 2. *Comparison of weights of Beta vulgaris, vars. Early Model and Egyptian Early, grown on plots "SW" and "E," the roots of which were inoculated with Bacterium tumefaciens; controls were growing among the inoculated plants. The inoculations were made August 4, 1920; the crop was harvested October 19, 1920*

No.	Plants grown in Plot "SW" (unfertilized soil)				Plants grown in Plot "E" (fertilized soil)			
	Normal		Root Inoculated		Normal		Root Inoculated	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
1	5.90	.50	24.30	1.90	22.20	1.55	11.50	1.10
2	3.70	.45	17.50	1.50	27.60	2.15	33.10	3.90
3	5.30	.65	2.50	.20	18.80	1.20	38.40	3.10
4	5.50	.65	8.40	.70	47.2	3.70	21.10	2.05
5	9.50	1.45	19.40	1.50	24.30	2.10	29.30	25.05
6	7.60	.80	9.80	.70	17.20	1.70	61.60	5.30
7	9.00	1.00	8.50	.60	28.30	2.70	17.10	1.20
8	23.80	2.30	16.80	1.50	27.10	2.60	56.10	4.60
9	29.80	3.80	5.00	.60	28.80	7.70	38.10	3.90
10	9.00	1.30	8.10	.60	19.60	4.20	38.00	3.80
11	24.00	3.30	10.90	.90	54.50	3.00	26.00	2.70
12	14.00	1.40	5.00	.50	37.50	2.50	24.10	1.50
13	5.40	.90	4.30	.40	42.20	.90	44.00	2.70
14	4.50	.50	7.20	.80	9.10	2.25	96.10	6.50
15	2.40	.30	7.90	.70	23.40	1.55	20.10	1.40
16	6.00	.60	5.20	.50	21.70	2.80	42.10	3.25
17	11.20	1.30	6.80	.65	34.60	1.00	33.50	2.40
18	39.00	3.50			13.00	5.40	32.50	4.50
19	9.00	1.90			79.20	3.50	83.10	7.00
20	10.00	.90			46.80	3.40	25.70	2.70
21	8.00	1.70			20.60	1.10	18.10	1.50
22	12.90	1.90			18.00	2.90	37.60	2.90
23	20.00	.45			33.50	6.10	71.50	6.00
24	20.50				69.50	.70	21.80	1.70
25	4.60				9.70		18.50	1.65
26							72.10	6.50
27							28.50	2.45
28							36.10	2.60
29							16.00	1.10
Totals	301.50	31.55	167.60	14.25	774.40	66.10	1,091.60	115.05
Averages	12.06	1.26	9.85	.83	30.96	2.64	37.64	3.96

The average weight of the entire plants when fresh is 12.06 g. The average dry weight is 1.26 g. The uninoculated plants grown in plot "E," that is, in fertilized soil, attained an average weight of 30.96 g. with a dry weight of 2.64 g. (columns 5 and 6). The difference in the weights of the normal plants can be interpreted only as due to differences in the amounts of available food in the soil.

The uninoculated plants in both plots were, as noted, scattered among those that had been inoculated. The average weight of the inoculated plants grown in unfertilized soil (plot "SW") is less than that of the healthy plants (see table 2, columns 3 and 4). This difference in favor of the normal plant indicates that the presence of the parasite in these cases has lowered the total growth (tissue-producing capacity) of the plant.

A slight variation in the number and size of the leaves affects the total weights of the plants, yet the beet roots of the same variety bearing crown galls are always larger and weigh more than the normal roots grown under similar conditions. The marked difference in weight between the inoculated plants grown in plot "E" (fertilized soil, see table 2, columns 7 and 8) and the inoculated plants grown in plot "SW" is of considerable interest and not only indicates a difference in the nutritive conditions of the plants but confirms the data already obtained for these plants when grown in pots, and also supports the contention that the healthy plant responds to the influence of an invading organism more vigorously than does the poorly nourished or less robust plant.

These results further support the view maintained by many plant and animal pathologists that the response to an invading organism is greatest and that the parasite is most favored when the necessary metabolic processes of the host are satisfied.

The Weight of the Crown Gall Compared with the Weight of the Plant

I have further studied the weight of the crown-gall tumors from both the large and the small beets. The varieties used were Early Model, Egyptian Early, and Giant Mangel Wurzel. The plants in this experiment were grown under the most favorable conditions. When the tap roots had developed they were inoculated with *Bacterium tumefaciens* on August 4, 5, and 6, 1920, in the manner described above and were harvested October 15, 1920. An equally large number of plants among the inoculated ones were left to serve as controls. Figure 8 represents a portion of plot "W" in which the Giant Mangel Wurzel was growing.

For convenience, the crown gall was considered as that part of the root which could be separated from it by a stroke of the knife, continuous with the normal contour of the root. It is readily seen that this does not remove all the crown gall, for the neoplasia in the plant also invades the normal tissues, as Levin and Levine (1920) have shown for other plants, so that

the weights of the crown galls in tables 3 and 4 are somewhat below the actual weights; while the weights of the roots given in these tables are above the actual weights of the normal root tissue.

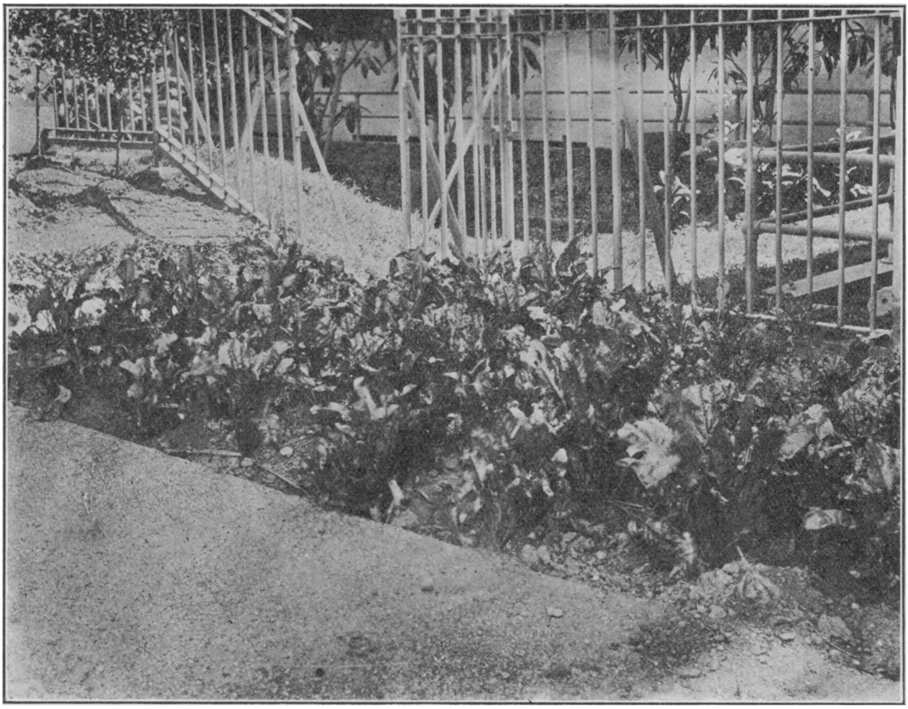

FIG. 8. A portion of plot "SW" showing the mangel wurzel, one month after the roots were inoculated with *Bacterium tumefaciens*.

The weight of the crown gall was determined by subtracting the weight of the plant, with the crown gall removed, from the weight of the entire plant. The weight of the leaves was determined by subtracting the weight of the root from that of the plant minus the crown gall.

The normal or uninoculated garden beet, varieties Early Model and Egyptian Early, showed an average weight of 39.49 g. (see table 3). The weight of the average normal root is 11.98 g., while that of the crown is 27.51 g. The entire weight of the average inoculated plant was 32.94 g., which is slightly below the normal weight. The average weight of these plants with the crown galls removed is 23.69 g., and the average weight of the crown galls is 9.25 g. The root without the crown gall averaged 8.61 g., the average weight of the crown gall and root is 17.86 g., which is above the average weight of the normal or uninoculated root. The decrease in size of the crown in the inoculated plants explains the difference in weight between the normal and the inoculated plants. I have not undertaken at

this time to explain the cause for the difference in these crowns. It may be suggested that possibly the disturbance of the vascular tissues of the root by the crown gall may be responsible for it, but no definite evidence is at hand.

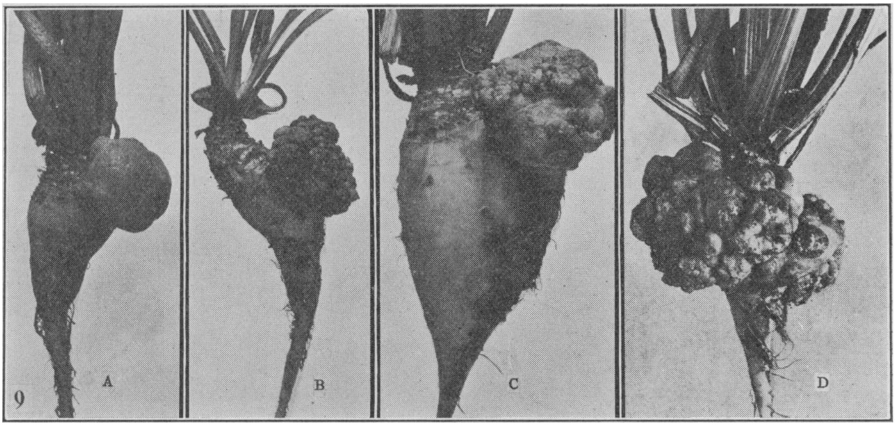

FIG. 9, A, B, C, D. Types of crown gall on the mangel wurzel: A, smooth; B, warty; C, smooth-warty condition combined; D, small smooth galls covered with clusters of warts.

The weights of the inoculated and uninoculated mangel wurzel show similar conditions. The average weight of the uninoculated mangel wurzel grown in these cultures is 113.47 g. The weight of the root is 52.09 g., and the average weight of the crown is 61.38 g.

The average weight of the inoculated plants, samples of which are shown in figure 9 with the smooth, warty, and mixed galls, is 124.38 g. (see table 4), which is far above the average weight of the normal plant. The weight of the plant without the mass of the crown-gall tissue is 84.31 g., while that part of the crown gall that could be removed from the root averaged 39.91 g. The average weight of the root is 41.69 g. and of the crown 42.78 g. The average weight of the root and its crown gall is 81.6 g. Here again the crown gall increased the weight of the root as in the other cases mentioned above; while the weight of the leaves in the inoculated plants was considerably lower, the growth of the root overcame the difference so that the average weight of the entire inoculated plants was greater than that of the normal plants.

It may be of further interest to note that the crown galls in the mangel wurzel, harvested approximately two months after inoculation, weighed more than the remainder of the root. None of the crown gall tissue on the mangel wurzel had disintegrated at the time they were harvested, but on being brought into the laboratory and moistened, decay set in at once. The crown galls alone turned black, and within 48 hours they were disintegrating while the normal tissues of the root remained unchanged.

TABLE 3. *Weights of the leaf, root, and crown gall of Beta vulgaris, varieties Early Model and Egyptian Early, compared with the weight of the entire plant when grown under favorable conditions*

No.	Normal			Root Inoculated August 15, 1920		Harvested October 20, 1920		
	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
1	32.50	8.30		18.60	13.80		5.00	
2	137.70	33.80		52.50	30.60		6.80	
3	32.20	5.10		56.20	52.50		18.40	
4	10.00	3.00		19.00	11.70		5.60	
5	20.70	7.00		48.00	22.70		13.20	
6	19.10	2.50		108.80	100.90		18.00	
7	13.90	3.70		75.30	59.70		20.20	
8	22.20	2.20		57.40	47.80		23.40	
9	20.50	4.60		20.20	7.30		4.20	
10	16.70	3.00		13.90	13.00		2.10	
11	24.00	5.00		20.00	13.50		6.70	
12	13.50	4.20		30.00	19.50		6.00	
13	14.20	2.30		98.75	75.50		18.90	
14	160.00	29.70		27.50	15.30		6.10	
15	49.70	14.30		9.40	5.60		2.20	
16	71.70	13.90		23.60	15.60		5.10	
17	36.80	4.10		29.60	16.10		8.40	
18	15.90	2.90		93.00	64.30		35.50	
19	69.00	85.00		122.00	105.00		57.40	
20	9.50	5.00		72.20	54.20		17.85	
21				26.70	17.60		4.10	
22				17.80	9.00		2.90	
23				20.80	17.60		3.80	
24				37.10	30.70		6.90	
25				33.50	28.20		6.90	
26				18.40	11.80		14.40	
27				29.60	10.85		4.20	
28				17.60	9.60		6.50	
29				32.60	18.50		3.80	
30				42.70	37.30		5.70	
31				11.80	9.90		6.70	
32				23.00	20.30		2.90	
33				14.00	7.80		2.90	
34				19.30	15.90		1.60	
35				15.10	6.70		3.00	
36				59.00	47.60		3.70	
37				40.10	25.10		16.20	
38				38.40	16.30		18.00	
39				42.50	23.30		4.50	
40				31.30	22.50		6.70	
41				9.60	8.60		6.50	
42				11.70	11.00		4.70	
43				135.50	121.00		4.50	
44				20.90	18.20		19.90	
45				35.60	31.00		10.10	
46				38.00	29.00		16.00	
47				18.10	0.00		8.90	
48				45.60	24.30		3.50	
49				13.65	19.70		14.70	
50				46.60	34.10		15.40	
51				656.85	11.30		8.80	
52				21.50	24.90		5.95	
53				36.00	477.80		10.70	
54				13.70	9.60		175.05	

No.	Normal			Root Inoculated August 15, 1920		Harvested October 20, 1920		
	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
55				18.80	23.70		3.50	
56				23.50	8.00		5.80	
57				13.80	14.60		4.50	
58				12.80	12.00		7.80	
59				13.40	9.00		3.90	
60				22.05	8.90		4.00	
61				11.80	8.10		1.80	
62				37.50	17.35		3.30	
63				25.35	8.80		8.50	
64				12.90	27.40		3.40	
65				15.30	15.80		20.50	
66				15.30	9.50		2.70	
67				11.70	9.30		1.80	
68				17.70	11.10		3.40	
69				20.10	8.30		2.40	
70				15.50	9.50		4.00	
71				15.80	19.00		4.30	
72				18.00	8.60		4.20	
73				392.50	7.30		3.70	
74					9.70		1.45	
75					255.55		4.70	
76							99.65	
Total	789.80	239.60	550.20	2,405.30	1,729.70	675.60	628.65	1,101.05
Averages	39.49	11.98	27.51	32.94	23.69	9.25	8.61	15.08

Discussion of Results

The average weight of the root of the normal beet grown in fairly fertile soil is less than the average weight of the root of the same variety bearing a crown gall due to an artificial inoculation with *Bacterium tumefaciens* and grown under similar conditions. The average weight of the beets bearing crown galls and grown in pots filled with a garden soil and manure combination is greater than the average weight of the beets inoculated with *Bacterium tumefaciens* and grown in pots filled with sand. The average weight of the entire normal beet plants is greater or less than the average weight of the entire inoculated beet plant, depending upon the number of leaves on each. The leaves and crown of a normal beet plant weigh more than the leaves and crown of a plant of the same variety grown under similar conditions but whose root bears a crown gall. It is suggested that this difference in leaf development may be caused by interference with the distribution of food caused by the crown galls, although no direct evidence upon this question is available.

The evidence is clear that a well nourished, vigorously growing, and healthy host responds to the invasion of a parasite by a hypertrophy and a hyperplasia which are greater than result in the case of a poorly nourished or feebly growing host. These results are in agreement with the obser-

vations of other workers as to the relations of host and parasite in certain cases.

TABLE 4. *Relation of the weights of the leaf, root, and crown gall of the Giant Mangel Wurzel to the weight of the entire plant when grown under favorable conditions*

Normal				Roots Inoculated August 5, Harvested October 9, 1920				
No.	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
1	67.10	32.00	35.10	143.90	63.30		34.60	
2	123.40	68.10	55.30	50.30	33.70		28.70	
3	117.40	41.40	76.00	51.10	39.90		18.80	
4	102.80	37.40	65.40	45.70	21.30		14.20	
5	28.50	4.10	24.40	199.60	129.60		83.30	
6	76.60	14.30	62.30	250.80	170.30		80.20	
7	131.00	58.70	72.30	210.20	140.10		92.00	
8	81.05	55.60	25.45	135.90	96.80		42.90	
9	230.60	101.90	128.70	193.60	118.50		63.60	
10	114.40	38.50	75.90	51.10	36.40		21.10	
11	121.00	56.90	64.10	176.10	133.00		60.00	
12	226.30	144.50	81.80	163.70	76.60		37.80	
13	174.50	95.75	78.75	42.30	24.70		17.80	
14	75.00	35.50	39.50	111.80	60.80		22.20	
15	77.10	36.20	40.90	78.60	58.20		41.80	
16	82.40	32.30	50.10	85.60	61.05		39.10	
17	100.00	32.50	69.60	61.40	36.20		23.30	
18				58.90	40.80		26.00	
19				50.60	38.90		19.55	
20				86.40	38.70		22.70	
21				187.70	146.05		88.05	
22				86.70	78.40		32.70	
23				30.70	24.50		14.15	
24				38.20	37.70		16.10	
25				125.80	115.70		40.00	
26				190.00	116.35		73.55	
27				410.00	264.60		106.40	
28				31.05	25.80		15.20	
29				111.40	61.00		36.60	
30				152.90	109.00		43.50	
31				164.00	115.10		62.10	
32				121.30	89.20		51.00	
33				199.40	115.90		67.90	
34				83.35	57.10		28.00	
35				25.00	22.50		14.40	
36				110.60	73.00		44.30	
37				120.50	79.70		28.00	
38				32.40	24.00		13.00	
39				75.40	39.50		27.80	
40				209.40	142.30		47.00	
41				104.60	65.20		26.80	
42				57.40	34.70		27.00	
43				28.80	30.50		13.60	
44				178.00	84.00		65.50	
45				74.40	53.70		20.30	
46				25.60	13.20		2.80	
47				243.40	173.40		10.50	
48				211.50	186.00		122.00	
49				276.50	258.10		83.10	
50				154.50	135.30		43.85	
51				161.00	105.00		55.00	
52				200.90	137.70		97.05	

Normal				Roots Inoculated August 5, Harvested October 9, 1920				
No.	Total	Root	Leaf	Total	Without Gall	Gall	Root	Leaf
53........				51.50	46.40		14.00	
54........				118.40	64.20		38.70	
55........				68.70	40.50		24.00	
56........				137.60	103.10		42.10	
57........				129.00	73.80		43.60	
58........				198.60	123.40		62.90	
59........				165.50	90.00		28.10	
Totals.....	1,929.15	885.65	1,043.50	7,339.30	4,984.45	2,354.85	2,459.90	2,524.55
Averages ..	113.47	52.09	61.39	124.38	84.31	39.91	41.69	42.78

Spinks (1913) in his study of water and soil cultures of wheat and barley showed that these plants were more susceptible to *Puccinia glumarum* and *Erysiphe graminis* when the plants were provided with large amounts of available nitrogen. Plants which were semi-starved as regards nitrogen exhibited a considerable degree of so-called immunity. Peltier (1918) and Peltier and Frederich (1920) showed that in the case of citrus canker the hosts were more susceptible when placed in conditions that induced rapid and vigorous growth.

Fromme and Murray (1919), studying the angular leaf spot of tobacco, a bacterial disease, found that those factors which promote rapid and vigorous growth of the host favor the parasite, and again Thomas (1921), studying the relation of the health of the host *Apium graveolens* (celery) to infection with the fungus *Septoria apii*, observed that those conditions of temperature, feeding, etc., which favored the health of the host as evidenced by vigorous development also increased the number of infections on the leaves.

Crown gall is analogous to animal cancer, as maintained by Smith, Levin and Levine. In the case of animal cancer, Ewing holds that "good health appears to favor the growth of tumor grafts and poor conditions retard it," while Teague claims that he was able to transplant mammary carcinoma of dogs only in animals weakened with distemper. In this relation of host vigor to virulence of the disease, crown gall, potato wart, club root, and other plant diseases resulting in hypertrophy and hyperplasia which have not been studied from this point of view are analogous to animal cancer.

This does not argue for or against the parasitic origin of animal cancer, for while the end results in animal and plant cancers are analogous, the initial stimuli may be entirely different—parasitic, mechanical, or even chemical.

The correlative of this proposition is quite true also, for Miss Brown (1920) has showed that *Pestalozzia* sp. inoculated into Sapodilla may produce

a large swelling, apparently a crown gall, while in larch, hemlock, and blue spruce this organism produces blighting of the leaves and no galls.

Crown gall belongs in the great class of diseases involving hypertrophies and hyperplasias of the host tissue. The reaction of the host to the parasite in these cases is obvious and specific. In sharp contrast with these we have the great group of cases in which plants parasitized by fungous or bacterial parasites show susceptibility by lack of visible reaction. Their tissues become necrotic because of destruction by the invading parasite. Whether in the cases of crown gall, potato wart, club root, and many other diseases the reaction appearing in the form of neoplastic growths may be interpreted as actively protective, is a difficult question. The types of disease in the two cases are certainly sharply distinct in plants; the symptoms in the former indicating accelerated metabolic activity and growth, while in the second case we have necrosis such as the soft rots, dry rots, cankers, etc.

We need not accept the conception advanced by some plant pathologists that the better the health of the organism the greater the susceptibility to an invading parasite. Susceptibility implies a certain relative adaptation to serve as a substratum for the growth of the parasite. The data presented as to crown galls on beets do not bear on the question of susceptibility. Under the conditions described the beets all showed practically 100 percent susceptibility. It is, however, in my opinion clearly shown from the results described above that the invading parasite induces a visible reaction in direct proportion to the general health of the individual. The proliferation of cells in the region of the invasion depends upon the general vigor and capacity for growth of the host tissue. This is in marked contrast with those plant diseases in which disintegration of the tissues is the first obvious evidence of the presence of the parasitic germ.

Summary

1. *Beta vulgaris*, varieties Early Model, Egyptian Early, and Giant Mangel Wurzel, were grown in different kinds of soil to test the effect of the soils on the growth, size, and weight of the root when artificially inoculated with *Bacterium tumefaciens*.

2. Of pot cultures with (1) garden soil with an abundance of manure, (2) brown silt loam and manure, (3) brown silt loam, and (4) medium sand, the largest average weight of plants was obtained in the garden soil. The crown galls were also the largest on these plants. The plants grown in sand weighed the least, were the smallest in size, and had the smallest crown galls.

3. Beets grown in open-air plots gave the same results. Those on the better soil were larger and heavier, and the crown galls on these roots were larger than those on beets grown in the poorer soil.

4. While the weight of the individual plants both inoculated and unin-

oculated varied widely, the average weight of the roots with crown gall was greater than the average weight of the normal or uninoculated roots in the same plot.

5. Three different types of crown gall were observed in *Beta vulgaris*, namely, the smooth type, the warty type, and a mixed type consisting of the warty and smooth types.

6. The reaction of the beet to crown gall depends upon the health of the beet; that is, with any given lot of seeds the extent of the reaction and the size of the crown gall depend ultimately upon the condition of the soil and upon other environmental factors.

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